The Influence of Temperature and Humidity on Moulting Process of Immature Stages of Amblyomma lepidum (Acari: Ixodidae) Under Laboratory Conditions.

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ملخص البحث

تناولت هذه الدراسة تأثير الحرارة و الرطوبة النسبية على الدورة الحيوية للأطوار غير البالغة لقراد أمبليوما ليبدم تحت الظروف المعملية. درست عملية التطور عند درجات حرارة 27 م°، 35 م° و 40 م° حيث أن كل درجة حرارة مصحوبة بخمسة مستويات للرطوبة (97.8%، 85%، 75.5%، 51.2% و 32.4%). تحت 27 م° و نسبة رطوبة 97.8% كانت النسبة المئوية لإنسلاخ اليرقات 93.4% بينما عند درجة الحرارة كانت 100%. تحت رطوبة 85% قلت نسبة الإنسلاخ الى 31.1% و 43.6% تحت درجتى الحرارة 27 م° و 35 م° على التوالى. عند مستويات الرطوبة الثلاثة الأدنى ودرجتى الحرارة 27 م° و 35 م° لم تتمكن اليرقات من الإنسلاخ. الحوريات تمكنت من الإنسلاخ عند الحد الأدنى المستخدم من الرطوبة و هو 32.4% محققة نسبة تصل الى 99.4%. عند درجة الحرارة 40 م° و بغض النظر عن الرطوبة النسبية فإن كلا الطورين قد ماتا قبل بداية الإنسلاخ.

Summary

The effect of temperature and relative humidity (Rh) regimen on the biological cycle of the immature stages of *Amblyomma lepidum* was studied under laboratory conditions. The moulting process was studied at 27°C, 35°C and 40°C, with ultimate five sets of humidity (97.8%, 85%, 75.5%, 51.2% and 32.4%). At 27 °C and 97.8% Rh the mean per cent moult was 93.4%, while at 35°C under the same humidity, it was 100%. At 85% Rh, the moult percentage declined to 31.8% and 43.6% at 27°C and 35°C, respectively. At the three lower levels of Rh and temperature of 27°C and 35°C, no larvae were moulted. Nymphs at both 27°C and 35°C, succeeded to moult even at the lower end of Rh, reaching a mean of 99.4%. However, at 40°C regardless of the Rh, both larvae and nymphs died before the commencement of moulting.

Introduction

Hard ticks transmit a diverse group of viral, bacterial and protozoan disease agents (Goodman *et al.,* 2005). On the other hand, tick-borne diseases emerge and persist when the habitat is appropriate (Lubelczyk *et al.,* 2004; Elias *et al.,* 2006). *Amblyomma lepidum* is an East African three -host tick; the natural and important host for the adult is cattle (Hoogstraal, 1956; Yeoman and Walker, 1967; Pegram, 1979). Nymphs had been found on antelopes, bustards, domestic cattle and dogs. It is involved in the epidemiology of heartwater. (Karrar, 1960) and is incriminated as the vector of the disease in the Sudan. A successful breeding of *A. lepidum* on laboratory rabbits, was firstly reported by Mohamed *et al* (1992) who suggested that the humidity factor is more associated with the larvae pre-moulting death than the lethal factor in rabbit's blood.

This study was aimed to add more information pertinent to the life cycle and biology of *A. lepidum* and verifying the effect of different temperature and relative humidity sets on the development of the tick as a prerequisite for future control strategy.

Materials and Methods

Source and maintenance of ticks:

Amblyomma lepidum ticks used in this study were obtained from a laboratory colony reared in the Department of Entomology and Ticks, Central Veterinary Research Laboratories Centre, Khartoum, Sudan. Batches of larvae and nymphs were allowed to feed on rabbits according to Baily (1960). When dropped they were collected and counted according to the stage and prepared for the experiment.

Adjustment of temperature and humidity:

Three incubators adjusted to 27°C, 35°C and 40°C were used. For humidity control, five saturated salt solutions for each temperature level were prepared as recommended by Winston and Bates (1960). Potassium sulphate (K_2SO_4), Potassium

chloride (KCl), Sodium chloride (NaCl), Di-hydrated sodium chromate ($Na_2Cr_2O_72H_2O$) and Magnesium Chloride ($MgCl_2$) were used to adjust humidity to 97.8%, 85%, 75.5%, 51.2% and 32.4%, respectively.

Ticks testing:

Engorged larvae were collected on the day they dropped off the rabbits, distributed into 75 specimen tubes each containing 100 larvae and divided into three groups;(1, 2 and 3) and incubated at 27°C, 35°C and 40°C, respectively. Each group was further divided into five sub-groups of five tubes each corresponding with a set of relative humidity. The larvae were monitored daily till the end of the moulting. The pre-moulting and moulting periods were recorded. The moulting percentage (%) was calculated. The same procedure was followed for nymphs with the exception that each tube contained only 50 nymphs. Data was subjected to statistical analysis performing the means separation using Ryan Eino Gebriel Welsh multiple range test according to Day and Quinn (1989)

Results

Pre-moulting period and moulting success of engorged larvae:

Development of larvae at both 27℃ and 35℃ occurred at 85% and 97.8% relative humidity. At 27℃, the pre-moulting period increased with decreased humidity, while moulting period and moultability decreased with decreased humidity. At 35℃, both pre-moulting and moulting periods increased when relative humidity decreased. At both temperature ranges, moultability decreased with decreased relative humidity and ceased completely at 75.5% and below. The premoulting and moulting periods were found to decrease when temperature increased from 27 ℃ to 35℃ with the exception at 85% Rh. the moulting period increased (Table 1). None of the engorged larvae succeeded to moult at 40℃ under any level of humidity.

Table1: Mean±SD of premoulting, moulting periods and percent moultability of *Amblyomma lepidum* larvae at different sets of temperature and relative humidity under laboratory conditions.

Temp.		Relative Humidity (%)				
		97.8	**85**	**75.5**	**51.2**	**32.4**
27 °C	P.mp	22.2±1	24.8±3	0	0	0
	M.p	18.8± 2.6	13.8± 1.5	0	0	0
	% moult	93.4± 2.7	31.8±1..9	0	0	0
35 °C	P.mp	13±0.7	19 ±1.2	0	0	0
	M.p	6.2 ±0.8	17.6 ±2.4	0	0	0
	% Moult	100	43.6± 1.73	0	0	0
40 °C	P.mp, M.p, and moult %	0	0	0	0	

Temp.= temperatrure; P.mp= premoulting period; M.p.= Moulting period;% Moult = per cent moulted; ±= mean ±Standard Deviation

Pre-moultingPeriod and moulting success of engorged nymphs:

At 27℃, the effect of relative humidity on the development of nymphs was insignificant (P≥ 0.05). Nymphs moulted even at relative humidity of 32.4% and 51.2%. At 35℃ there was no fixed pattern of moulting in relation to humidity changes.

The pre-moulting period of nymphs at 35℃ was significantly shorter than that at 27℃ (P ≤0.001).On the other hand, moulting period increased at the higher ends of relative humidity and decreased at the lower ends. The mean percentage moultability at all conditions was above 93%. However, at 40℃, no nymphs moulted (Table 2).

Correlation analysis of data showed that larvae pre-moulting, moulting periods and moultability were positively correlated with humidity (p≤0.0001). The pre-moulting period was negatively correlated with temperature (p≤0. 0001), moulting period (p≤0.001), but moultability was less correlated (p≤0.05).

2: Pre-moulting, moulting periods and moulting per cent (Mean ±SE) of *Amblyomma lepidum* nymphs at different sets of temperature and humidity under laboratory condition.

Temp =temperature; P.mp = Premoulting period; M.p = Moulting period; % moult = Per cent moulted.

Temp℃	**Development periods in days and percent moult (mean ± SE)**					
		Relative humidity (%)				
	Character	97.8	85	75.5	51.2	32.4
27	P.mp	25.3± 0.8	25± 0.35	25.2± 0.57	24± 1.1	24.5± 0.3
	M.p	3.6± 0.5	3.4± 0.5	3.8± 0.8	3.6 ±1.1	3.8± 0.8
	% moult	97.6± 2.6	99.6± 0.9	(99.4 ±1.3	98 3±.08	99.4 ±1.3
35	P.mp	16.9 ±0.7	16.7± 1.35	15.2 ±0.76	17.1 ±0.8	16.2 ±0.9
	M.p	5.2 ±1.48	5.2±1.3	2.6 ±0.5	2.6 ±0.89	3.8± 0.4
	% moult	98.2 ±1.79	98.2 ±1.79	100	94.8 ± 0.84	98.4 ± 0.4
40	P.mp, M.p, and % moult	0	0	0	0	0

Discussion

This study was conducted under laboratory conditions utilizing sets of temperature and relative humidity simulating the Sudan conditions. Balashov (1967) stated that temperature is the factor that controls the moulting of both larvae and nymphs. The current study has revealed that larvae and nymphs develop well within 27°C and 35°C. However, at 40°C moulting was unsuccessful, determining the critical upper limit of temperature**.** This finding is in agreement with Mohammed (2002). Nevertheless, within this permissible range of temperature, the moulting of larvae was extremely affected by the increased saturation deficit (P ≤0.001). In contrast, nymphs unexpectedly resisted the saturation deficit and could moult even at the lower level of relative humidity. This finding may imply that this mid stage is the hardiest stage in the life cycle of the tick which seems to be quickly adaptable to the environmental changes. Virtually, it could be speculated that this stage in particular is responsible for maintaining the tick population at the field level. The pre-moulting period of larvae reported in this study is comparable to that of Mohamed *et al*, (1992) who reported 21 days and to that of Mohammed (2002) who reported a mean of 24.5 days under field conditions. Karrar *et al* (1963) found that larvae of *A. lepidum* could not moult at 80% relative humidity and Osman (1978) mentioned that larvae are unable to moult at any

temperature when humidity is below 85%, supporting the findings of the current study. However, Osman (1978) claimed that temperature range of 20℃ -30°C and relative humidity range of 90-95% being the optimum conditions for development of *A. lepidum*. In regards to nymph, Karrar (1968) reported a wide range of premoulting period which extended up to 47 days. Karrar *et al*, (1963) determined the temperature and relative humidity requirements of nymphs to be 22°C -27°C and relative humidity range of 60-80% .The premoulting period of both stages decreased when temperature was elevated from 27°C to 35°C. On the other hand, in this study increased saturation deficit resulted in prolonged premoulting period of larvae but the effect on nymphs was insignificant.

The moulting period of larvae at 35°C was comparatively shorter than that at 27°C. The same finding was reported for other tick species (Hussein and Mustafa, 1987; Pegram and Banda, 1990; ElGhali, 1992). The effect of relative humidity was, however, more pronounced. The moulting period of nymphs at the higher humidity concentration increased with the increase in temperature, while at the lower concentrations no definite relation could be drawn. At all laboratory conditions utilized, the moulting percent of nymphs exceeded 93% indicating the high tolerance of this stage. The laboratory findings of the current study support the findings of ElGhali and Hassan (2010) who have showed that, premoulting period and moulting per cent of *Hyalomma dromedarii* is affected by seasonality. However, it could be concluded that development of a definite combination of temperature and relative humidity for *A. lepidum*, is required.

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